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(54) **ANTI-HUMAN IMMUNODEFICIENCY VIRUS AGENT.**

(57) An anti-HIV agent containing as the active ingredient a polyfucose sulfate originating in a sea cucumber and having the following physicochemical properties and/or a pharmaceutically acceptable salt thereof: form: white, amorphous and highly hygroscopic powder; mol. wt.: about 20,000 ~ 200,000 (according to polyacrylamide gel electrophoresis); composition: hexosamine 1 to 7 wt. %, fucose 45 to 65 wt. %, sulfate group 25 to 42 wt. %.

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TECHNICAL FIELD

The present invention relates to a novel antiviral agent for human immunodeficiency virus (referred to as HIV in the specification) effective to HIV.

BACKGROUND ART

Acquired Immune Deficiency Syndrome (AIDS) is a serious immunodeficiency disease caused by HIV infection [Nature, 321, 10 (1986)], because of its high mortality, taking a counter-measure to the HIV infection and AIDS even becoming a great social problem.

Azidothymidine (AZT) having an inhibition activity of reverse transcriptase is known as an effective anti-HIV agent regarded as a clinically effective agent at present.

However, clinical effect of azidothymidine (AZT) used as an anti-HIV agent is not satisfactory enough, in addition azidothymidine has a problem that side-effects caused by AZT, for example damage of bone marrow (hematopoietic tissue), neurological complaints, such as headache, convulsion and the like are strong. In particular, in case of HIV, its genome infiltrates into a chromosome of an infected cell as a provirus, and the cell seems to exist in a condition of hereditary disease so that a long-term administration of a medicament for AZT is inevitably requested, such side-effects of AZT become a great difficulty when used as an anti-HIV agent.

Under the present condition, development of new pharmaceutical preparations having an effect on HIV infection and AIDS with small side-effects and being capable of long-term administeration are earnestly expected.

DISCLOSURE OF THE INVENTION

The present inventors conducted extensive research on compounds having an excellent effect on HIV, and found that polyfucose sulfates from a sea cucumber and/or pharmaceutically acceptable salts thereof have an excellent anti-HIV activity and are safe when administerd to patients in a long term, the present invention having been accomplished.

Thus, the present invention provides an anti-HIV agent containing a polyfucose sulfate from a sea cucumber and/or pharmaceutically acceptable salts thereof as an active ingredient.

The polyfucose sulfate from a sea cucumber used in the invention is a polyfucose sulfate (hereinafter referred to as PFS) extracted from the body wall of Holothurian and has the physicochemical properties shown below.

* Characteristics: white, amorphous and highly hygroscopic powder
* Molecular weight: about 20,000 to about 200,000 daltons (as measured by polyacrylamide gel electrophoresis method)
* Analysis for composition: composition by weight are as follows.

Hexosamine	1 to 7 wt.%
Fucose	45 to 65 wt.%
Sulfate	25 to 42 wt.%.

The following methods were used to analyze Hexosamine (hereinafter referred to as HexN), Fucose (hereinafter referred to as Fuc) and Sulfate.

* HexN
  Blumenkrantz method [Clin. Biochem., 9: 269 (1976)]
* Fuc
  Dische method [J. Biol. Chem., 175: 595 (1948)]
* Sulfate
  Dodgson method [Biochem. J., 78: 312 (1961)]

PFS is a known substance, described in, for example Yaoxue Xuebao, 1983, 18(3), 203-208; Science 1969, 166: 758-759; J. Biol. Chem., 1973, 248(1), 30-33; J. Biochem., 1972, 72, 1265-1267; Hirosaki Igaku, 1956, 7, 142-149 respectively, and can be easily produced by the methods described in them.

More specifically, PFS can be produced by extracting a body wall of Holothurian hydrolyzed with alkaline hydrolysis or enzymes, such as pancreatin and the like, followed by separation and purification of the extracts. Examples of sea cucumbers useful in the preparation of PFS are:

Stichopus japonicus Selenka,
Stichopus chloronoyus Brandt,
Stichopus variegatus Semper,
Holothuria pervicax Selenka,
Holothuria atra,
Holothuria argus,
Holothuria edulis,
Holothuria scabra,
Parastichopus nigripunctatus,
Thelenota ananas,
Holothuria monacaria Lesson,
Holothuria leucospilota Brandt,
Cucumaria chronhjelmi,
Cucumaria echinata,
Cucumaria frondosa Japonica,
Pentacta australis,
Paracaudina chilensis ransonneti,
Molpadia musculus,
Leptosynapta inhaerens,
Polycheira rufescens,
Synapta maculata,
Halodeima cinerascens(Brandt),
Actinopyga lacanora(Jaeger),
Actinopyga echinites(Jaeger),
Microthele nobilis(Selenka),etc.

The cucumber to be used as the starting material may be a raw or dried one. Of the cucumber exemplified above, Stichopus japonicus Selenka is most preferred as the starting material.

The above analytic results show that PFS has in the molecule sulfate group which reacts with bases to form a salt. These polyfucose sulfate are stable in the form of a salt and isolated and purified usually in the form of a sodium and/or potassium salt. These polyfucose sulfate salts can be converted into a free polyfucose sulfate by treated with cation exchange resins such as Dowex 50W (product of Dow Chemical Co.) and the like. Further, they can be converted into various desired salts by conducting conventional salt exchange when desired. Usable as salts of polyfucose sulfate are pharmaceutically acceptable salts including salts of potassium, sodium or like alkali metals, and salts of calcium, magnesium, barium or like alkaline earth metals, or pyridinium salt or like organic bases. The method for preparing PFS will be described below in detail in the following reference examples.

The polyfucose sulfates and/or pharmaceutically acceptable salts thereof are made into various pharmaceutical compositions useful for HIV treatment. Stated more specifically, the anti-HIV agents of the present invention comprising an effective amount of active ingredient of the invention and a pharmaceutically acceptable carrier can be prepared in various administration forms in accordance with various conditions of HIV treatment. The administration form can be any of tablets, capsules, powders, granules, grains, solutions, emulsions, suspensions and like oral forms, and injections, suppositories, semisolids, plasters and like parenteral forms. These preparations can be manufactured by conventional methods already known to those skilled in the art. A solid preparation for oral administration can be prepared by mixing the effective component of the invention with an excipient with or without addition of binders, disintegrators, lubricants, coloring agents, flavorings, perfumes, etc. and making them into tablets, capsules, powders, granules, grains or the like in a conventional manner. Injection preparations can be produced by adding a pH-adjusting agent, buffer, stabilizer, isotonizing agent, localanesthetic and the like to the effective component of the invention, and making the mixture into intravenous, intramuscular, subcutaneous, intracutaneous or intraperitonial injections in a conventional manner. Suppositories can be prepared by making a mixture of the effective component, base materials and optionally a surfactant and the like into a suppository in a conventional manner. A semisolids preparation can be prepared by adding base materials, a pH-adjusting agent, a lubrication-auxiliary agent, optionally adding antiseptics, preservatives and the like when required, to the effective component of the invention and making the mixture into ointment, cream, jelly and the like in a conventional manner, preferably used as a lubricant.

Examples of excipients useful as for oral solid preparations are lactose, sucrose, starch, talc, magnesium stearate, crystalline cellulose, methyl cellulose, carboxymethyl cellulose, glycerin, sodium arginate, gum arabic, etc. Examples of binders useful as for oral preparations include polyvinyl alcohol, polyvinyl

ether, ethyl cellulose, gum arabic, shellac, sucrose, etc. Examples of useful lubricant are magnesium stearate, talc and the like. The coloring agents, disintegrators and other auxiliaries to be added include those commonly used in the art. Tablets may be coated by well-known methods.

Examples of base materials useful as for suppositeries include oily base materials such as macrogol, lanolin, cacao oil, fatty acid triglyceride, Witepsol (registered trademark for the product of Dynamite-Nobel AG) and so on.

Examples of base materials useful as for semisolids preparations include carboxymethyl cellulose, sodium carboxymethyl cellulose and the like gelationizing agent. Examples of pH-adjusting agent useful as for semisolid preparations include sodium hydroxide, aqueous ammonia etc. Examples of useful lubrication-auxiliary agents are polyethylene glycohol, polyvinyl alcohol, carboxymethyl cellulose, sodium arginate, polyacrylic acid and the like. Examples of useful antiseptics and preservatives are paraoxybenzoate esters (methyl, ethyl, propyl and butyl ester) etc.

The amount of the effective component per each unit dosage varies with the symptoms of the patient to be given the preparation, the form of the preparation, etc. Usually a preferred amount is 10 to 200 mg in an oral preparation, 1 to 100 mg in a injection and 10 to 100 mg in a suppository, per each unit dosage. The daily clinical dosage of the composition of the invention also varies with the patient's age, sex, conditions and other factors but usually may be in the range of about 10 to about 1,000 mg, preferably about 50 to about 200 mg in terms of the effective component per day and can be given at 1 to 4 divided doses.

As a lubricant, the present agent can be used for prevention of HIV infection by appling the agent to the vulva or the penis before a sexual act.

BEST MODE FOR CARRING OUT THE INVENTION

The present invention will be described in greater detail with reference to Reference Examples, Examples and Pharmacological Tests. The percentages in Reference Examples and Examples are all by weight.

REFERENCE EXAMPLE 1

Preparation of PFS-1

Starting material was prepared by pounding 4 kg of fresh Stichopus japonicus Selenka whose viscus had been removed after washing with water, followed by dehydration with ethanol treatment to give dried powder. To a predetermined quantity of the powder was added 50 times as much water as the powder by weight, and the mixture was stirred overnight at room temperature while maintaining pH of the solution at about 8 by adding diluted sodium hydroxide solution. An insoluble material was removed by centrifugation and filtration. The removed insoluble material was further extracted two or three times in the same condition as above. Obtained extracts were combined and dialyzed against water, and a formed precipitate was filtrated. A 1 % of benzidine hydrochloride solution was added to the filtrate until any precipitate was not formed. The formed precipitate was collected and washed with 0.1 % of benzidine hydrochloride solution. The precipitate was suspended in water and stirred for 2 hours while maintaining pH of the solution at 8 by adding diluted sodium hydrochloride solution to remove benzidine which was separated from the solution. After the filtrate was neutralized and concentrated, a small quantity of potassium acetate and ethanol 4 times as much volume as the residue was added to the residue. A formed precipitate was collected by centrifugation, washed with ethanol and ether in this sequence and dried to give 7.9 g of a crude product.

A 500 mg portions of this crude product was dissolved in 50 ml of 0.02 M sodium phosphate buffer (pH 6.5) containing 1 M sodium chloride and purified with DEAE-Cellulose (product of Sigma Chemical Co.) column (2.5 x 50 cm). Elution was conducted in conditions using as a eluate 0.02 M sodium phosphate buffer (pH 6.5) containing sodium chloride increasing the concentration of sodium chloride from 1M to 3M at a flow rate of 30 ml/h. The fractions whose sodium chloride concentration was 2 M were collected, dialyzed against water and lyophilized to give 47 mg of extract from 500 mg of the crude product. A 100 mg of the extract prepared by repeating the above procedure was dissolved in 10 ml of 0.02 M sodium phosphate buffer (pH 6.5) containing 1 M sodium chloride and purified by the same procudere as above. The fractions whose sodium chloride concentration was 2 M were collected, dialyzed against water and lyophilized to give 47 mg of extract from 100 mg. This extract was dissolved in 5 ml of 0.02 M sodium phosphate buffer (pH 6.5) containing 1 M sodium chloride and purified with DEAE-Cellulose column (1.4 x 27 cm). The elution was conducted in conditions using as a eluate 0.02 M sodium phosphate buffer (pH 6.5) containing sodium chloride and increasing the concentration of sodium chloride from 1M to 2M at a flow rate of 10 ml/h. The

fractions whose sodium chloride concentration was 1.5 M were collected, dialyzed against water and lyophilized to give 28 mg of purified product from 47 mg. The analytical values of the purified product are as follows.

Molecular weight: about 170,000 to about 180,000 daltons (as determined by polyacrylamide gel electrophoresis method)
Fucose: 59.5 %
Sulfate: 32.7 %
Hexosamine: 1.83 %
Uronic acid: 0.13 %
Pentose: 1.04 %
Protein: 1.00 %
Ash: 22.2 %.

REFERENCE EXAMPLE 2

Preparation of PFS-2

Dried Stichopus japonicus Selenka was pulverized, and lipid was removed with acetone. A 2 kg of this dried and delipidized Stichopus japonicus Selenka was put into a 13.6 1 of 0.2 M potassium acetate solution, and a 16 g of Protin A (product of Daiwa Fine Chemical Co.) which is a protease was added to the mixture and the resulting mixture was stirred at 70 °C for 1 hour. A 15 g of Protin A was added again to the solution and the resulting mixtere was stirred at 70 °C for 2.5 hours. After removing an insoluble material by centrifugation, a 320 g of sodium chloride was added, ethanol being added until the concentration of ethanol became 20 %, and the mixture was allowed to stand overnight to remove a precipitate formed by centrifugation. To the supernatant saturated cetylpyridinium chloride solution was added until any precipitate was not formed, the mixture being allowed to stand overnight to collect a precipitate formed and the collected precipitate was washed with water. This precipitate was dissolved in 1680 ml of ethanol with heat, and the solution was cooled to room temperature to collect a precipitate formed. This precipitate was dissolved in 2 l of n-propanol with heat, and the solution was cooled to room temperature to collect a precipitate formed. This precipitate was dissolved in 1600 ml of n-propanol : water (2:1) solution with heat, and the solution was cooled to room temperature. The precipitate formed was removed by centrifugation, and to the supernatant was added 1 % (W/V) of potassium acetate to collect a precipitate formed. This precipitate was washed with ethanol and acetone in this sequence, and dried under reduced pressure to give 34.2 g of a crude product. This crude product was dissolved in a 855 ml of 1M sodium hydroxide solution containing 2 % of sodium chloride. To this solution was added dropwise a 85.5 ml of hydrogen peroxide solution (30 %), and the mixture was stirred at 60 °C for 6 hours. The solution was cooled to room temperature, formed insoluble material being removed by centrifugation, and the pH of the solution was adjusted at 6.5. To the solution was added ethanol until the concentration of ethanol became 35 %, and the mixture was cooled to 4 °C. The precipitate formed was removed by centrifugation, and ethanol was further added to the supernatant until the concentration of ethanol became 56 %. The mixture was cooled to 4 °C to collect a precipitate formed by centrifugation. The precipitate was dissolved in 220 ml of water, and the solution was added dropwise to 1100 ml of ethanol containing 0.5 % of potassium acetate. The mixture was cooled to 4 °C to collect a precipitate formed by centrifugation. The precipitate was washed with ethanol and acetone in this sequence, and dried under reduced pressure to give 24.1 g of purified product. The analytical values of the purified product are as follows.

Molecular weight: about 100,000 to about 110,000 daltons (as determined by polyacrylamide gel electrophoresis method)
$[\alpha]_D^{20}$: -199.78°
Fucose: 53.6 %
Hexosamine: 5.1 %
Uronic acid: 0.4 %
Sulfate: 35.6 %
Protein: 1.7 %
Sodium: 5.7 %
Potassium: 6.4 %.

REFERENCE EXAMPLE 3

Preparation of PFS-3

A 5.3 g of PFS having the following physicochemical characteristics was produced by the same procedure as Reference Example 2.

Molecular weight: about 120,000 to about 130,000 daltons (as determined by polyacrylamide gel electrophoresis method)
$[\alpha]_D^{20}$: -204.23°
Fucose: 53.3 %
Hexosamine: 4.2 %
Uronic acid: 0.3 %
Sulfate: 32.6 %
Protein: 0.8 %
Sodium: 5.0 %
Potassium: 9.6 %.

REFERENCE EXAMPLE 4

Preparation of PFS-4

The dried body wall of Holothuria leucospilota Brandt was cut in piece, hydrolyzed in potassium hydroxide solution, and the mixture was neutralized with acetic acid. The mixture was filtrated and concentrated. The concentrate was allowed to stand in a refrigerator overnight to give a transparent solution of supernatant by centrifugation. To the solution was added ethanol twice as much volume as the solution to give precipitate. The precipitate was dissolved in proper amount of water, and the solution was subjected to oxidized decolorization. To the decolorized solution was added ethanol twice as much volume as the solution again to give crude product. The crude was dissolved in 2 M potassium chloride solution in an amount of 1:30 (W/V), and the solution was allowed to stand in a refrigerator overnight to remove a precipitate by centrifugation. To the transparent solution of the supernatant was added the same volume of hexadecylmethylammonium bromide solution, and the precipitate was decolorized by wash and precipitation with 1 M potassium chloride using Celite-545 (product of Wako Pure Chemical Ind.) as a filtration auxiliary and subsequently washed with 1.5 M of sodium chloride to conduct dissociation. A small quantity of the solution was dispenced, ethanol being added to the solution to give a precipitate, and agarose electrophoresis (hexadiamine buffer) was applied to the precipitate to give a dissociated solution. Ethanol was added to the solution to give a precipitate. After hydrolyzing the precipitate, ethanol was added again to give a precipitate. The precipitate was washed with ethanol and acetone, dehydrated and dried to give purified product. The analytical values of the purified product are as follows.

Molecular weight: about 84,000 daltons (as determined by polyacrylamide gel electrophoresis method)
$[\alpha]_D^{20}$: -151.3°
Fucose: 51.0 %
Hexosamine: 2.7 %
Sulfate: 29.3 %.

Each polyfucose sulfate obtained in the above Reference Examples 1 to 4 showed a simple spot on electrophoresis [Dietrich C. P., J. Chromatogr., 130, 299 (1977)].

EXAMPLE 1

Injection

PFS sodium potassium salt produced in Reference Example 2 was dissolved in water for injection to give a 5 % water solution. This solution was packed in a vial for lyophylization in an amount corresponding to 50 mg of a polyfucose sulfate from Stichopus japonicus Selenka per one vial, and lyophylization was conducted. A 2 ml of physiological saline for dissolution was attached to the bial separately.

PFS sodium potassium salt (Reference Example 2)	40 mg
physiological saline	proper quantity
per 1 ample	2 ml

EXAMPLE 3

Tablet

A tablet was prepared according to the following prescription.

PFS sodium potassium salt (Reference Example 2)	10 mg
cornstarch	65 mg
carboxymethyl cellulose	20 mg
polyvinylpyrrolidone	3 mg
magnesium stearate	2 mg
per 1 tablet	100 mg

EXAMPLE 4

Suppository

A suppository was prepared according to the following prescription.

PFS sodium potassium salt (Reference Example 2)	50 mg
Witepsol W-35 (product of Dynamite-Nobel AG)	950 mg
per 1 piece	1000 mg

	W/V %
PFS sodium potassium salt (Reference Example 2)	0.3-0.5
carboxyvinyl polymer	0.1
polyethylene glycol	10.0
1 N-sodium hydroxide	proper quantity
perfume	proper quantity
purified water	proper quantity
	total 100 ml

Polyethylene glycol and PFS sodium potasium salt were dissolved and stirred in 10 ml of purified water in this sequence, and then carboxyvinylpolymer was dispersed in the solution. After pH of the solution being adjusted at 6.3 using 1N-sodium hydroxide, and purified water was added to the solution to give a total volume of 100 ml, a perfume was added and the solution was treated with an autoclave at 100 °C for 20 minutes.

PHARMACOLOGICAL TEST

(1) Anti-HIV Activity

MT-4 cells [Miyoshi I. et al., Gann Monogr., 28, 219-228 (1982)] were adjusted to privide a 2 x 10^5 cells/ml medium (RPMI-1640 + 10 % FCS). To 500 µl of the medium were added 50 µl of a solution of sea cucumber-derived polyfucose sulfate obtained in Reference Example 2 in varying concentrations. Two hours after the addition, the cells were infected by HIV in a proportion of 5 x 10^3 Pfu/well. Four days later, a smear of MT-4 cells was formed, and HIV antigen positive cells were determined by fluorescent antibody technique [Harada S. et al., Science, 229, 563-566, (1985); Takeuchi, Y. et al, Jpn. J. Cancer Res.(Gann), 78, 11-15 (1987)].

A proportion of virus-infected cell (HIV antigen positive cell) is shown in Table 1 below.

Table 1

PFS concentration of Reference Example 2 (µg/ml)	10	1	0 reference
Infected cell proportion (%)	0	10	95

The above results show that the anti-HIV agent of the invention inhibit infection of HIV effectively.

(2) cytotoxicity

The cytotoxicity of polyfucose sulfate from sea cucumber was determined in accordance with the above (1). Detailedly, After MT-4 cells prepared in a concentration of 1 x 10^5 cells/ml were cultured for 4 days in the presence of sea cucumber-derived polyfucose sulfate obtained in Reference Example 2 in varying concentrations, the number of the cells being counted, cell survival ratios in each concentration being calculated when the cell survival ratio of reference cell is 100 %, and cytotoxicity was evaluated. The result are shown in Table 2 below.

Table 2

PFS concentration of Reference Example 2 (μg/ml)	100	10	1	0 reference
Infected cell proportion (%)	105	105	104	100

The above results show that the anti-HIV agent of the invention has no significant cytotoxicity.

INDUSTRIAL APPLICABILITY

The anti-HIV agent of the present invention has an effect on prevention of HIV infection, prevention of crisis of AIDS and AIDS related complex [ARC; R.R.Redfield & D.S.Burke, Science, 18, 74-87, (1988)] and therapy thereof and can be suitably used in a long-term administration because of the low cytotoxicity of the active ingredient, i.e., polyfucose sulfate from sea cucumber.

Therefore, the anti-HIV of the present invention has an effect on prevention of HIV infection to uninfected people, prevention of crisis of carrier and therapy thereof and is very effective to cure a patient of AIDS and ARC.

Claims

1. An anti-HIV agent containing a polyfucose sulfate from sea cucumber having the following physicochemical properties and/or pharmaceutically acceptable salt thereof as an active ingredient:

Characteristics: white, amorphous and highly hygroscopic powder

Molecular weight: about 20,000 to about 200,000 daltons (as measured by polyacrylamide gel electrophoresis method)

Composition by weight:

Hexosamine	1 to 7 wt.%
Fucose	45 to 65 wt.%
Sulfate	25 to 42 wt.%.

2. An anti-HIV agent according to claim 1 containing a polyfucose sulfate from sea cucumber having the following physicochemical properties and/or pharmaceutically acceptable salt thereof as an active ingredient:

Molecular weight: about 170,000 to about 180,000 daltons (as measured by polyacrylamide gel electrophoresis method)

Composition by weight:
Hexosamine: 1.83 wt.%
Fucose: 59.5 wt.%
Sulfate: 32.7 wt.%
Uronic acid: 0.13 wt.%
Pentose: 1.04 wt.%
Protein: 1.00 wt.%
Ash: 22.2 wt.%.

3. An anti-HIV agent according to claim 1 containing a polyfucose sulfate from sea cucumber having the following physicochemical properties and/or pharmaceutically acceptable salt thereof as an active ingredient:

Molecular weight: about 100,000 to about 110,000 daltons (as measured by polyacrylamide gel electrophoresis method)

$[\alpha]_D^{20}$: -199.78°
Composition by weight:
Hexosamine: 5.1 wt.%
Fucose: 53.6 wt.%
Sulfate: 35.6 wt.%
Uronic acid: 0.4 wt.%
Protein: 1.7 wt.%
Sodium: 5.7 wt.%
Potassium: 6.4 wt.%.

4. An anti-HIV agent according to claim 1 containing a polyfucose sulfate from sea cucumber having the following physicochemical properties and/or pharmaceutically acceptable salt thereof as an active ingredient:

Molecular weight: about 120,000 to about 130,000 daltons (as measured by polyacrylamide gel electrophoresis method)
$[\alpha]_D^{20}$: -204.23°
Composition by weight:
Hexosamine: 4.2 wt.%
Fucose: 53.3 wt.%
Sulfate: 32.6 wt.%
Uronic acid: 0.3 wt.%
Protein: 0.8 wt.%
Sodium: 5.0 wt.%
Potassium: 9.6 wt.%.

5. An anti-HIV agent according to claim 1 containing a polyfucose sulfate from sea cucumber having the following physicochemical properties and/or pharmaceutically acceptable salt thereof as an active ingredient:

Molecular weight: about 84,000 daltons (as measured by polyacrylamide gel electrophoresis method)
$[\alpha]_D^{20}$: -151.3°
Composition by weight:
Hexosamine: 2.7 wt.%
Fucose: 51.0 wt.%
Sulfate: 29.3 wt.%.

6. Use of a polyfucose sulfate from sea cucumber having the following physicochemical properties and/or pharmaceutically acceptable salt thereof to prepare a pharmacological composition for treating diseases caused by HIV.

Characteristics: white, amorphous and highly hygroscopic powder

Molecular weight: about 20,000 to about 200,000 daltons (as measured by polyacrylamide gel electrophoresis method)
Composition by weight:

Hexosamine	1 to 7 wt.%
Fucose	45 to 65 wt.%
Sulfate	25 to 42 wt.%.

7. Use according to claim 6 wherein said polyfucose sulfate and/or pharmaceutically acceptable salt thereof has the following physicochemical properties:

Molecular weight: about 170,000 to about 180,000 daltons (as measured by polyacrylamide gel electrophoresis method)
Composition by weight:
Hexosamine: 1.83 wt.%
Fucose: 59.5 wt.%
Sulfate: 32.7 wt.%

Uronic acid: 0.13 wt.%
Pentose: 1.04 wt.%
Protein: 1.00 wt.%
Ash: 22.2 wt.%.

8. Use according to claim 6 wherein said polyfucose sulfate and/or pharmaceutically acceptable salt thereof has the following physicochemical properties:

Molecular weight: about 100,000 to about 110,000 daltons (as measured by polyacrylamide gel electrophoresis method)
$[\alpha]_D^{20}$: -199.78°
Composition by weight:
Hexosamine: 5.1 wt.%
Fucose: 53.6 wt.%
Sulfate: 35.6 wt.%
Uronic acid: 0.4 wt.%
Protein: 1.7 wt.%
Sodium: 5.7 wt.%
Potassium: 6.4 wt.%.

9. Use according to claim 6 wherein said polyfucose sulfate and/or pharmaceutically acceptable salt thereof has the following physicochemical properties:

Molecular weight: about 120,000 to about 130,000 daltons (as measured by polyacrylamide gel electrophoresis method)
$[\alpha]_D^{20}$: -204.23°
Composition by weight:
Hexosamine: 4.2 wt.%
Fucose: 53.3 wt.%
Sulfate: 32.6 wt.%
Uronic acid: 0.3 wt.%
Protein: 0.8 wt.%
Sodium: 5.0 wt.%
Potassium: 9.6 wt.%.

10. Use according to claim 6 wherein said polyfucose sulfate and/or pharmaceutically acceptable salt thereof has the following physicochemical properties:

Molecular weight: about 84,000 daltons (as measured by polyacrylamide gel electrophoresis method)
$[\alpha]_D^{20}$: -151.3°
Composition by weight:
Hexosamine: 2.7 wt.%
Fucose: 51.0 wt.%
Sulfate: 29.3 wt.%.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP91/01013

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl[5] A61K31/725

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	A61K31/725, C08B37/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	JP, A, 63-10601 (Seikagaku Kogyo K.K.), January 18, 1988 (18. 01. 88), (Chemical Abstracts Abstract No. 109(10): 79691b) (Family: none)	1-15
A	JP, A, 63-45223 (Ueno Seiyaku Oyo Kenkyusho K.K.), February 26, 1988 (26. 02. 88), & EP, A, 871007 & AU, A, 8771074 & US, A, 4840941 & JP, A, 1151521	1-15
A	JP, A, 63-128001 (Taiho Pharmaceutical Co., Ltd. and another), May 31, 1988 (31. 05. 88), (Chemical Abstracts Abstract No. 110(10): 82475k) (Family: none)	1-15
P	WO, A, 90-8784 (Taiho Pharmaceutical Co., Ltd.; Kotai Kasei Co., Ltd.), August 9, 1990 (09. 08. 90), (Chemical Abstracts Abstract No. 114(16): 150158u) & AU, A, 9050325 & EP, A, 408770	1-15

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
October 1, 1991 (01. 10. 91)	October 21, 1991 (21. 10. 91)
International Searching Authority	Signature of Authorized Officer
Japanese Patent Office	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

	& JP, X, 2-502725	
P	WO, A, 90-9181 (Taiho Pharmaceutical Co., Ltd.; Kotai Kasei Co., Ltd.), August 23, 1990 (23. 08. 90), (Chemical Abstracts Abstract No. 114(12): 108909q) & AU, A, 9050389 & EP, A, 410002 & JP, X, 2-503019	1-15
A	Chemical Abstracts Abstract No. 109(22): 196956c (Gekkan Yakuji, 29(6), 1167-1174(1987) Kitakawa Isao: "Biologically active	1-15

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers , because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

•	marine natural products.")	
A	Chemical Abstracts Abstract No. 97(22): 18841b (Zhongyao Tongbao, 7(4), 27-29(1982) Fan, Huizeng; Chen, Judi: "A new method for the isolation and purification of an acidic mucopolysaccharide from Stichopus japonicus")	1-15
A	Chemical Abstracts Abstract No. 95(16): 138509r (Yao Hsueh T'ung Pao, 16(4), 58(1981) Chen, Chu-Ti; Fan, Huei-Tseng; Wen, Yu-Lin:	1-15

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	"Observations on the stability of said mucopolysaccharide potassium salts from Stichopus Japonicus")	
A	Chemical Abstracts Abstract No. 94(8): 52763m (Fan, Hui-Zeng; Chen, Ju-Di; Lin, Ke-Zhong: "Isolation of mucopolysaccharide from Stichopus japonicus selenka and some of its physical and chemical properties.")	1-15

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